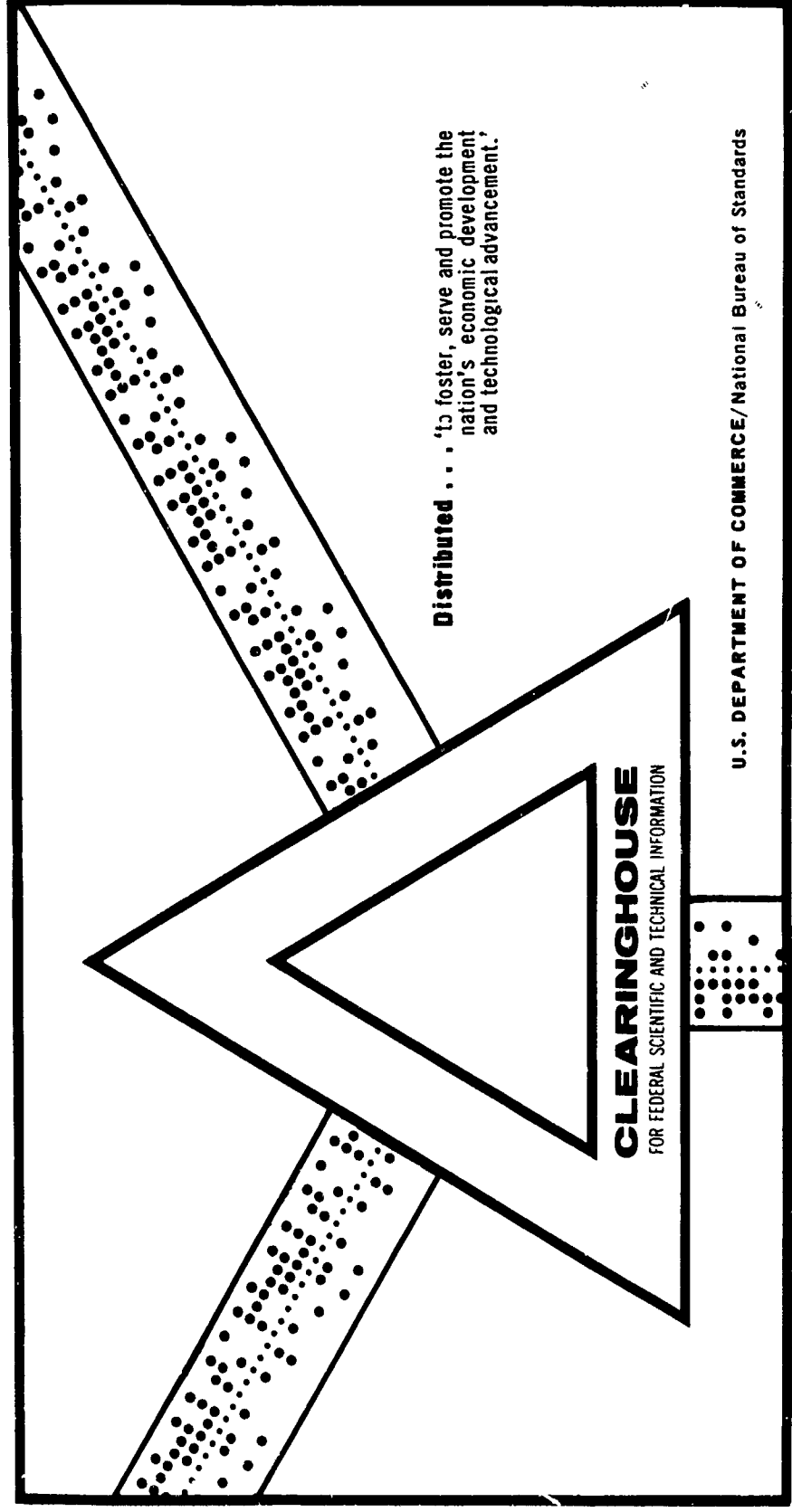


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SELECTED CONTRIBUTIONS TO THE LITERATURE OF BLOOD GROUPS AND
IMMUNOLOGY. VOLUME III, PART 2. M N AND P SYSTEMS

Frank R. Camp, Jr., et al
Army Medical Research Laboratory
Fort Knox, Kentucky

17 May 1969



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**SELECTED CONTRIBUTIONS TO THE LITERATURE OF
BLOOD GROUPS AND IMMUNOLOGY**

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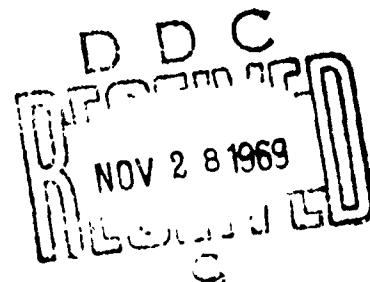
**Vol. III
in two parts**

**Part 2
M N and P Systems**

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**Blood Transfusion Division
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121**

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May 1969

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PURPOSE

This series of fundamental research reports from the field of blood group immunology has been prepared for the Fellowship in Blood Banking and Immunohematology for career military personnel. The translation of these works was prepared by the Frank C. Farnham Company, Philadelphia, Pennsylvania. Certain prime English reports have also been republished because of their extremely limited availability.

ACKNOWLEDGMENTS

We wish to thank the following individuals for their arduous contributions in such tasks as finding and obtaining permission from publishers, editors, authors and next of kin; typing, proofreading and distribution; and finally for the myriad of correspondence generated in such an effort:

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Very special recognition must go to Mr. Richard A. Wheeler for photographic support and technical advice.

PREFACE

We have included four of the original papers suggested to us by Ivor Dunsford during the early formulation and selection of articles for the Translation Series. These are Crome's hereditary study of M and N, another genetic study in the same system by Friedenreich, and two papers by Dahr on the heredity of P.

In addition, we have also placed the book Constitutional Serology and Blood Group Research by Ludwig Hirszfeld in Volume III. The role of "this very group-specific differentiation of the blood" was having an impact on the physician, the biologist, anthropologist, and geneticist, in the 1920's. Hirszfeld saw that the sudden popularity of blood groups in applied and pure science was, in one sense, desirable but also held many pitfalls. Therefore, Hirszfeld hoped that this book would fulfill a two-fold goal: to present that which is already known and considered certain, and to point forward to that which may possibly be achieved in the future.

The Editors

Fort Knox, Kentucky

Lt Colonel Frank R. Camp, Jr., MSC

Detroit, Michigan

Colonel Frank R. Ellis, MC (USAR)

17 May 1969

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QUESTIONS PERTAINING TO BLOOD GROUPS:
MOTHER M, CHILD N

W. Crome

Translation of "Über Blutgruppenfragen: Mutter M, Kind N."
Dtsch. Z. ges. gerichtl. Med. 24: 167-175, 1935.

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US ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX KENTUCKY 40121

QUESTIONS PERTAINING TO BLOOD GROUPS:
MOTHER M, CHILD N*

W. Crome

In our regular blood group determinations, we had, while ^{**/167} investigating a parenthood question, the opportunity to investigate a very remarkable case several times. Because of the blood group of mother and child, with regard to the characteristics M and N, this case seems important enough to be reported in some detail.

The district physician responsible for the case sent us sterile blood taken from the unwed mother and the reported father in Behring venules, and from the illegal child in capillaries, all blood having been taken correctly and labeled properly. Investigations showed that all persons belonged to the blood group O, and that, on the basis of agglutination and absorption tests the reported father belonged to blood group MN, the mother to blood group M and the child to blood group N.

Since, according to Landsteiner's inheritance theory, a pure M parent cannot be the progenitor of a pure N child, we assumed that the blood of the mother and of the father had been inadvertently exchanged. We asked the district physician for another blood sample from the three persons, with the added request to again make sure that he was dealing with the correct persons and to take all possible precautions against an accidental exchange of the blood samples.

The blood testing, which was repeated after several months, yielded the same results. According to agglutination and absorption tests, the mother belonged to blood group M and her illegal child to blood group N.

To eliminate all possible sources of error, to exclude possible mistaken identity or even substitution of the child, and in order to be able to investigate the blood taken freshly from both mother and child, the home district of the parents had both mother and child come to ^{/168} our Institute for a blood test. This third blood test again took place

*Read at the 22nd Meeting of the German Association for Legal and Social Medicine in Hanover, September, 1934.

**/Numbers in the margin indicate pagination of the original foreign text.

after several months, and this time we could examine the persons ourselves, have them photographed, etc. Since the mother of the unwed mother had also come, we had the opportunity to test her blood also. The grandmother indicated that the unwed mother was her own legal child. According to the indications of the unwed mother, which were confirmed by the information in her file, her child had been born at home, thus excluding the possibility of a substituted child.

The unwed mother was a 19-year old, strong and healthy girl, with no history of past illness, and her son, at that time 1 1/2 years old, was at a normal stage of development.

All three persons belonged to blood group O, and the reactions were in accordance with Landsteiner's rule.

As in the two previous examinations, which had been conducted according to all the rules of the art, we found again that the mother of the child belonged phenotypically to blood group M and the boy to blood group N. According to the agglutination tests, the grandmother belonged to blood group N.

Although all the results of the tests which were taken at different times were in complete agreement, I thought it necessary to have further controls done by other investigators with specialized experience. Therefore, while taking blood for our Institute, I took further samples from mother and child and sent them to Thomsen (Copenhagen), Lauer (Hamburg) and Mayser (Stuttgart), with a request for investigating the samples. I would like to thank these gentlemen for reporting their findings to me.

My own investigations of the M and N characteristics were done by using the object-carrier method, taking the final reading of the agglutination results after a 15 minute waiting period. This method was supplemented by the centrifuging method, with a reading after a five minute waiting period and centrifugation for two minutes, at 1500 revolutions.

I used 4 anti-M sera and 7 anti-N sera. These sera were freshly prepared before the third test, they were especially carefully cleaned and tested as to specificity and titer strength. They were still found pure, after the object-carrier and centrifuge method was applied, using the experimental conditions described above. The anti-N working sera were tested with special care; they did not react with M test blood corpuscles, their specific titer, as compared to pure N test blood was not

lower than 1:16, measured from the serum dilution before adding /169 an equal quantity of blood corpuscle suspension, and a reading taken after the centrifuge method.

The qualitative agglutination tests were further supplemented with quantitative agglutination tests after the centrifuge method. In the different anti-M working sera, we still found clear agglutination with the blood corpuscles from the mother, with sera dilutions not below 1:32 to 1:128, whereas the anti-N working sera did not lead to any clumping. In the blood of the child, the anti-M working sera did not lead to any clumping, whereas the anti-N working sera clumped this blood, in dilutions not below 1:16 to 1:32. These investigations agreed quantitatively with control experiments with pure N and M test blood investigations.

The absorption experiments were also done with the various working sera, absorbing, in the usual way, 0.3 cc of working serum with 0.1 cc washed red blood corpuscles, twice per 1/2 hour, at room temperature. Repeated tests always yielded the same results, i. e., the blood of the mother bound the M-factor completely. With the various anti-N working sera, the blood of the mother caused a weakening of the titer by about 1 1/2 to 2 dilution stages, still corresponding to what, according to various experiences, could be expected during experiments with M blood. Simultaneous absorption-control investigations with M test blood yielded the same results and control investigations with MN test blood led to complete binding of the M and N antibodies.

Further, 2 raw anti-N sera, in a dilution of 1:40 with 1/4 volume (each) of washed red blood corpuscles of the mother were absorbed twice, each time for 1/2 hour, at room temperature. The poured-off remainder proved to be free of M test blood corpuscles and it had a specific titer, as compared to the N blood of the child, of 1:16 to 1:32. Simultaneous control experiments, using absorption of both raw anti-N sera with M test blood, yielded the same results.

Further details need not be mentioned at this point, especially not the investigation of the blood of the child, since according to the uniform results even of the control investigators, the blood of the child was, without a doubt, pure N blood.

Lauer reported the results of his investigations, which also had been taken with all possible precautions about 48 hours after the blood sample had been taken, indicating that according to the results of the

agglutination and absorption tests, the blood of the mother must belong to blood group M and the blood group of the child had to be N. He remarked that he had found the M-factor of the mother was less able to bind, as for example a mixed-inherited M-factor; however, this could have been due to the fact that the blood was not quite fresh. /170

The investigations in the Württemberg State Investigative Bureau were conducted by Dr. Haug, who has been a colleague of Dr. Mayser for many years. She reported that the samples were tested about 24 hours after they had been taken, using agglutination tests with the object-carrier and centrifuge method, each time using several anti-M and anti-N working sera. Further, absorption experiments had been undertaken as well with the raw sera, as with previously treated anti-N sera. According to the results of these investigations the mother had the characteristic M and the child the characteristic N. Mayser later reported that, in the object-carrier experiment with an anti-N working serum, a very slight clumping of the red blood corpuscles of the mother could be observed, a fact which, considering the completely negative results of all other tests, was not an indication to doubt the lack of N characteristics.

Thomsen investigated the blood samples by using agglutination tests, among others with two different anti-N working sera, about 30 hours after the blood had been taken. He found very weak agglutination of the blood corpuscles of the mother, which was only recognizable after about 20 minutes, even in the case of the anti-N working serum that showed a somewhat stronger agglutination than the other one. The absorption experiments showed that the blood corpuscles of the mother bound the anti-N bodies, very weakly, and lowered the agglutination titer by about 1 1/2 to 2 dilution stages, whereas the control investigation with M test blood caused a weakening of the titer by about 1 dilution stage, and whereas controls with MN blood bound all of the anti-N. Even the absorption of the M antibodies by the blood of the mother was somewhat weaker than that of the MN control blood.

Professor Thomsen had V. Friedenreich investigate the blood of the mother four days after it had been drawn, in the meantime keeping it refrigerated. Several anti-N working sera gave negative agglutination tests, corresponding with a repeated test by Thomsen on the same day. With one anti-N working serum he observed slight agglutination. All the absorption tests done by Friedenreich had negative results.

On the basis of the results of his tests, Thomsen indicated that the blood of the mother belongs to the MN type, however, with a very

weakly developed M-receptor, in fact, so weak that it could remain /171 undetected and might not have been detected if one would have known from the type of the child that the mother had to have an N-receptor.

To this I would like to remark that I did not give Lauer and Mayser any indications as to the origin of the blood samples, whereas Thomsen could see from my report that it had to be the blood of a mother and her child.

Summarizing these results, we can see that all investigators agree that the blood group of the child certainly is N, i. e., it lacks the M-factor.

Three investigators indicated that the blood of the mother belonged to group M, while Thomsen believes that it must be blood group MN, however, with very weak and possibly, under certain circumstances (such as settling), undetectable N-receptor.

Landsteiner, to whom I reported the various investigation results, agreed with me insofar as the N-characteristics in the mother had not been established with certainty, however, what seemed important to him was to prove the absence of N; this proof was not fully established.

Initially, I believed that I could shed more light on the case with immunization experiments on rabbits, using the blood of the mother, thus possibly establishing the presence of the N-characteristic in the mother indirectly, by proving the presence of a specific N antibody in the immune serum.

In investigations which have not been published, we succeeded, through immunization of rabbits with serum of the blood group OM and ON, to clearly show the formation of anti-M or anti-N bodies in the immune serum. Apparently, a very slight antigenic stimulus suffices to cause a formation or further concentration of specific antibodies because the quantitative receptor content of the blood serum is so small. For instance, in the agglutinin binding experiment the detection of receptors M and N is impossible, whereas, as mentioned, it is possible to detect them in the immunization experiment.

Landsteiner has already pointed out in his report (J. exp. med. 47: 770, 1928) that he has occasionally observed the formation of N antibodies during immunization with M blood, however, never the formation of N antibodies during immunization with N blood. I investigated

my raw sera with the same thought in mind, and I obtained the same results. In nearly all raw anti-M sera from different immunization series, whereby each series is made from the OM blood of one donor, and the different series are made from the M blood of different donors, traces of specific N reactions could still be detected after absorption. In several cases these specific N reactions showed an average titer strength /172 of 1:4 to 1:8, as compared to pure N blood.

Landsteiner considers it to be possible that "rabbits could possibly develop small quantities of antibodies without any specific stimulus (?)". Since, according to this, during immunization experiments with the blood of the mother, the formation of specific anti-N bodies is probably no proof for the phenotypical presence of the characteristic N in the mother, I have, for these and other reasons, done without these considerations.

It is obvious, according to the test results on the grandmother, mother and child, that the disagreement with the inheritance theory of Landsteiner, as it pertains to blood group characteristics M and N, must stem from the mother of the child. But even if we assume, as I believe is the case, that the mother belongs phenotypically to blood group M and her child to blood group N, the available investigative material, which pertains to the inheritance of M and N characteristics, would still corroborate the "correctness" of Landsteiner's theory (only considering its practical useability) and could not be doubted because of one unusual case. Without a question, the mother of the child must be of the genotype that is carrier of the characteristic N, since she must have inherited this from her pure N mother and must have transferred it to her child.

If we merely focus our attention on the practical significance of our case and, if possible, neglect all theoretical considerations, it is rather insignificant whether we consider the blood of the mother to be M blood or, as Thomsen, MN blood with a very weak N-receptor, and it is also unnecessary to consider the deviation of the test results and their possible causes.

This case seems to teach me that in rare cases, one must consider the possibility of so-called "phenotypical latency". In the mother, for example, the characteristic N in the phenotype, for unknown reasons, has not asserted itself, a possibility which is not generally proven for the various blood group characteristics and which may not be demonstrable at all, but which is known to exist in the general theory of genetics and which Thomsen considers possible, for example,

for the characteristic A₂. To this it must be remarked that the investigative material is very small which deals with the inheritance of all blood group characteristics known so far, especially with so-called critical cases, when we compare it to investigative material dealing /173 with plants and animals, which makes it possible to experiment and to crossbreed.

Furthermore, if we follow the test results of Thomsen then we must expect exceptional cases, this case teaches us where a characteristic (in our case the N-receptor) is developed to such a small extent that the usual methods do not serve to detect it at all, or only very uncertainly.

In this context I would like to report a case which was investigated by B. Mueller in Göttingen and which was placed at our disposal. On the basis of agglutination tests, a very experienced serologist had found a blood sample to be pure M-blood. Mueller had already found in repeat blood studies that this blood gave a changing but still clear reaction, although very weak, with anti-N working sera and he thus began to doubt the correctness of the M diagnosis.

Our own repeat studies showed that the characteristic N of this blood could clearly be shown by agglutination and absorption tests. However, the strength of all reactions, measured quantitatively, was considerably lower than is usually found with MN blood, i. e., the N-receptor of this blood was only very weak. Another repeat study was intended, but had to be abandoned for external reasons. In the meantime, Mueller informed me that the same blood was being studied in the Thomsen Institute in Copenhagen, with the same results, i. e., agglutination tests with anti-N working sera yielded very weak reactions which led to a diagnosis of M or possible MN. In the absorption tests this blood bound all the M antibodies and almost all N antibodies, and was therefore diagnosed as an MN type. It was assumed as probable that the direct N agglutination test was very weak, because the agglutinability of the blood was probably weakened through storage.

Further, Mayser informed me about a case which he intended for repeat studies where the characteristic N was very weak, although detectable.

Landsteiner informed me that he was not at all surprised at the occurrence of weak N reactions, and that he had reported such cases in his first writings; he referred me to a table in that report (J. exp. med. 47: 760, 1928, Table 1).

If we summarize the results of our observations for legal medicine concerning questions of parenthood, independently of all theoretical deliberations, we find the following significant conclusions:

In practice it is rather unimportant in our case whether we consider the factor N to be clearly detected in the blood of the mother or not.

Without a doubt, our observations teach us that in spite of perfect technique, we must consider the possibility that especially in the blood group MN, the characteristic N which phenotypically is to be expected, may not be detectable or may be so weak that the investigator may overlook it.

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According to available investigative material, we may assume that these are very rare exceptions.

In cases where, in spite of intensive and exact methods, there are still some deviations from the normal results and hence doubts as to positive or negative N reactions, i. e., when it comes to the decision whether we are dealing with M blood or MN blood or vice-versa, the final report should be very cautious as to the decision of definite parenthood.

The detection of positive or negative M reactions, i. e., the diagnosis of N or MN, does not pose any particular difficulties.

A prerequisite for successful work in investigation of the blood group characteristics M and N, seems to be the continuous working with exact methods in suitably equipped laboratories, where the working sera are manufactured on location, thus making continuous control possible.

I do not have any practical experience concerning the useability of so-called ready-to-use working sera, which are commercially available, however, I know from other investigators that there is disagreement about the useability, particularly of anti-N sera. For a person without much serological experience there certainly is a certain danger in using these sera, because of misunderstanding and underestimating the difficulties of the investigative techniques.

Final evaluation of blood groups for legal purposes is not formed after complete exhaustion of a not-quite uncomplicated method, nor without continuous control of the sera with a great number of test bloods

as to specificity and titer strength. Neither is evaluation performed by the omission of control investigations in all cases where there is any doubt at all; even the possible omission of absorption tests. Such errors can only contribute to unjustifiably miscredit this branch of blood group investigation.

Our case points to the absolute necessity to continue to investigate families, especially mother-child pairs, as to their hereditary factors. Suitable investigative material can be obtained from clinics for females and from obstetrical clinics. I was able to examine about 1200 mother-child pairs, without finding one deviation from the rule. It would further be appropriate if all investigators would publish investigative material, which often is probably kept secret.

The practical value and the significance of blood group characteristics M and N, as they concern legal questions of parenthood, cannot be significantly diminished by one deviating observation. In pertinent cases, even this part of blood group science is, and must be /175 in the future, a very significant indication whose value must be considered much higher than the many consciously or unconsciously erroneous or unreliable statements of witnesses, a phenomenon which is especially apparent in matters related to questions of parenthood.

The degree to which the investigator believes he is able to decide with "certainty" or with "very great probability" a question of legal fatherhood from the blood group characteristics M and N, must rest with each investigator, depending on his sense of responsibility and his estimation of biological knowledge.

Without investigating the unfortunate concept of "obviously impossible", it is an impossible task to demand absolute certainty from biological investigative methods.

The natural limitation of these circumstances will not change in the future, although it would be possible and it is to be hoped that in a future form of jurisprudence the legal requirements will be brought into agreement with scientific knowledge and possibilities.

It seems to me to be the task of legal medicine to furnish preparative work and cooperation.

**A GENETICALLY DEFECTIVE N-RECEPTOR, POSSIBLY
INDICATING A SO-FAR UNKNOWN BLOOD GROUP
CHARACTERISTIC WITHIN THE MN SYSTEM**

V. Friedenreich

Translation of "Ein erblicher defekter N-Receptor, der
wahrscheinlich eine bisher unbekannte Blutgruppeneigenschaft
innerhalb des MN-Systems darstellt." Dtsch. Ztschr.
Gerichtl. 24: 358-368, 1936.

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**US ARMY MEDICAL RESEARCH LABORATORY
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A GENETICALLY DEFECTIVE N-RECEPTOR, POSSIBLY
INDICATING A SO-FAR UNKNOWN BLOOD GROUP
CHARACTERISTIC WITHIN THE MN SYSTEM*

V. Friedenreich

In the course of a blood group determination performed to **/358 determine a question of fatherhood, we discovered a blood group constellation of mother and child which seemed to be in contradiction with the genetic rules of the MN system.

The mother appeared to belong to group M and the child to group N, a combination which should not be possible since an M mother, according to the system, should only have M or MN children; being homozygotic, she must give M to her child.

We obtained new blood samples, and repeated tests on the persons concerned, using selected, strong, fresh sera indicated a very weak reaction to the strongest anti-N sera on the part of the mother. Thus, she really belonged to group MN, although with defective N-receptor development.

After that, we obtained blood samples from some of the nearest relatives, i. e., the mother and two of the mother's sisters. We observed identical characteristics in all three. Thus, we were not dealing with an isolated case of defective receptor development, but with the familiar occurrence of an abnormally weak N-receptor.

The characteristic serological findings made on these individuals are apparent from the examples listed in the following tables, because all adult carriers of the characteristic, when compared directly, proved to be identical.

Table 1 shows the effect of 10 different anti-N sera blood corpuscles of one of these persons as compared to normal MN and M blood corpuscles.

The 10 sera belong to 4 different series obtained through immunization of rabbits with blood corpuscles from four different N individuals.

*Lecture before the 6th Nordic Pathology Congress, Cslo, July, 1955.

**/Numbers in the margin indicate pagination of the original foreign text.

The reaction was carried out by mixing serum and a suspension of blood corpuscles on a slide. The reading was taken after 10 minutes.

TABLE 1.

/359

Blood Corpuscles	Anti-N Serum Number									
	1	2	3	4	5	6	7	8	9	10
D. H.	0	0	0	(+)	+	+	+(+)	+	0	+(+)
MN	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
M	0	0	0	0	0	0	0	0	0	0

We see that only 5 of the 10 sera give a D. H. reaction with blood corpuscles, although very weak. All sera, however, yield maximum agglutination of normal MN blood corpuscles.

Those sera which do react positively are normally the strongest (measured by titration as compared to normal blood corpuscles).

Table 2 explains the difference in the speed of reaction. After 5 minutes, the reactions of normal MN blood corpuscles reaches a maximum, whereas the atypical ones give only very insignificant traces of reactions. Only after another 5 minutes, we can observe clearly positive reactions in the strongest sera.

TABLE 2.

Blood Corpuscles	Anti-N Serum Number							
	After 5 minutes				After 10 minutes			
	5	6	7	8	5	6	7	8
D. H.	Trace	(+)	(+)	(+)	(+)	+(+)	+(+)	+(+)
MN	+++	++++	++++	++++	++++	++++	++++	++++
M	0	0	0	0	0	0	0	0

Table 3 shows titration of the receptor strength.

For practical reasons, the titration was done according to the glass slide method. The reading was taken after 10 minutes. As is

known, this method yields rather low titer values (at least 2 degrees lower than the centrifuge method). At the same time this method yields very weak reactions, comparatively clear but which are only detectable in the strongest serum concentrations.

From this we can see that the sensitivity of the atypical N-receptor is about 1/8 of that of the normal N-receptor.

TABLE 3.

Blood Corpuscles	Serum Dilution					
	1/1	1/2	1/4	1/8	1/16	1/32
D. H.	+(+)	(+)	0	0	0	0
MN	++++	++++	++	+	0	0
N	++++	++++	+++	++	(+)	0
M	0	0	0	0	0	0

The results of the absorption experiments are also very re- /360 markable. Using the usual routine technique (with only qualitative reading of the result), we could never detect agglutinin binding (e. g., not even during the first test on the mother of the child, where the diagnosis for M was confirmed through absorption). Only with a very favorable adjustment of the ratio between the quantity of absorption blood corpuscles and the serum strength, and by quantitative reading of the results, can we see a certain effect (Table 4a). In Table 4b, where the serum has been diluted somewhat less, we can see no difference in the absorption effect between D. H. and M blood corpuscles.

TABLE 4.

	a					b				
	1/1	1/2	1/4	1/8	1/16	1/1	1/2	1/4	1/8	1/16
Anti-N Serum, unabsorbed	++(+)	+(+)	+	0	.	+++	++	+(+)	(+)	0
Anti-N Serum, absorbed with										
1/2 vol. of										
blood cor-										
puscles										
(a) = Trace										
D. H.	+	(a)	0	.	.	+++	++	(+)	0	.
M	++	++(+)	(a)	0	.	+++	++	+	0	.
N	0	(+)	0	.	.	.
MN	0	+	0	.	.	.

Prof. O. Thomsen was kind enough to investigate some of these blood samples. In the first investigation, using the object-carrier method, he found rather strong, but slow reactions in the anti-N sera and in attempting titration to determine the receptor sensitivity, using the test tube method, no reaction was observed. Absorption experiments yielded no detectable agglutinin binding. In the second investigation of the same persons, the slide glass method yielded reactions from $++$ to $+$. No absorption effect.

When we were sufficiently familiar with the serology of the weak N-receptor, we investigated its occurrence in the family of the mother, a farmer family, which was found to be healthy in all respects.

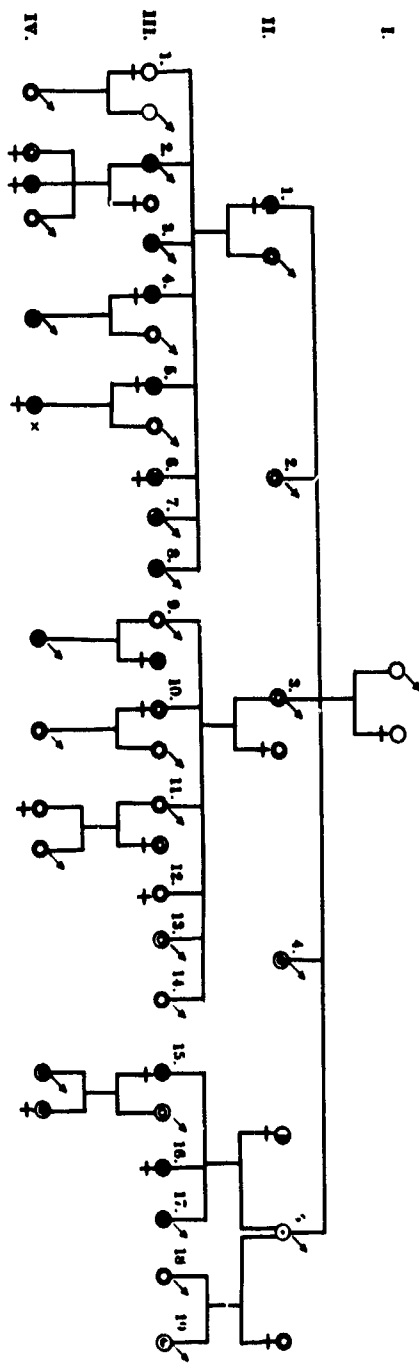
The blood samples were tested on the same day they were taken. The diagnosis "weak N" could be established immediately in all cases by testing with a positively and negatively reacting serum. All MN blood corpuscles were tested with all 10 sera, the speed of reaction was noted and the sensitivity of the N-receptors was measured by means of titration. As we said before, the quantitative investigations showed that all individuals had the same weak N characteristic (we will report on two children at a later time).

The results can be seen from the family tree (see illustration). (The weak N-receptor is designated N_2 , the normal receptor N_1 . The child which was the starting point of our investigation is designated as X.)

It is to be added that all M-receptors are completely normal /361 and that the receptors designated N_1 do not deviate in any aspect from control N (or MN) blood corpuscles.

The predisposition for N_2 can be veiled by N_1 , although it does not occur in either M or MN_1 ; the N_1 tendency does not occur with N_2 (MN_2). This process of inheritance can only be explained in the following way: the weak N characteristic stems from an independent allelomorph gene (designated N_2), joined to M and N_1 , which is, however, dominated by N_1 , similar to the relationship of A_2 to A_1 in the ABO system.

(Any other hypothesis to explain this inheritance process if it is at all applicable, becomes complicated and unreal when compared /362 to the simple and natural supposition of another gene from the allelomorph series.)



1. An N_2 parent has, except with an N_1 partner, only N_2 , but no N_1 descendants (II, 1).
2. An N_1 parent may have N_2 descendants (III, 15).
3. An MN_1 parent, with an M partner has only MN_1 , but no MN_2 descendants.
4. An M parent of the family has no N_2 descendants (II, 3).

Legend: ● = M ; ● = N_1 , ● = MN_1 ; ● = MN_2 ; ○ = dead, or not investigated for other reasons; ⊙ = dead, reconstructed: N_2 ; ⊙ = dead, reconstructed: N_1 .

The family tree shows the realization of all the important genetic combinations in this system. From the parent combination $MN_1 \times MN_2$ we must have descendants of e.g., MN , MN_2 , MN_1 and N_1N_2 . As we can see, the first three combinations can be found in the children of (III, 2) and the fourth one in the X child and in (III, 15) whose N_2 characteristic (covered up by N_1) is demonstrated by the MN_2 children with the M husband.

The MN system thus includes 6 genotypically different and, probably, 5 phenotypical classes (the last one has never been observed):

<u>Genotype</u>	<u>Phenotype</u>
MM	M
MN_1	MN_1
MN_2	MN_2
N_1N_1	} N_1
N_1N_2	
(N_2N_2)	N_2 [?]

The extension of the MN system is completely analogous to the amplification of the ABO system, which was occasioned by the discovery of the A_2 group. The peculiarity of the N_2 characteristic is its rarity, and since it is not easily detectable it assumes practical significance of quite another kind. However, this circumstance does not alter the conception of the biological nature of the characteristic; from a biological standpoint, the N_2 characteristic, since it depends on an independent allelomorphic gene, constitutes a group characteristic,* irrespective of its rarity.

Further analogies with the A_1A_2 system should be mentioned briefly. It is known that the B-receptor in the A_1B group is usually somewhat weaker than in the pure B group, whereas the B-receptor in A_2B groups is not influenced by the B component. Correspondingly, the M-receptor in MN_2 has almost always been found to be as strong as in pure M, whereas the MN_1 it is known to be somewhat weaker.

*Whether N_2 is to be designated a group or a sub-group (of N), is, as in the case of the A distinction, a matter of opinion, depending on whether the distinction is made according to genetic or to serologic viewpoints.

As far as the serological basis for the difference between the N_1 - and the N_2 -receptor is concerned, we are confronted with the same possibilities as in the A_1A_2 question; whether the low sensitivity of the N_2 -receptor to anti-N is due to quantitative or qualitative deviation /365 from the N_1 -receptor, has to remain unanswered for the time being.

The practical significance of the N_2 -receptor is the fact that, since it can escape detection, it may lead to erroneous blood group determination (diagnosis M instead of MN_2).

The fact that it can be overlooked when using sera which, so far, were considered to be good, is apparent from the above considerations. The question remains whether we can be sure of detecting it with sera which meet the highest standards achievable with the present technique.

The answer to this question is doubtlessly a negative one. Even when the typical N_2 -receptor gives a strong reaction with the strongest N sera that we know today, the titer will still be extremely low and we must be prepared for dire consequences, which are caused, in part, by the inevitable variations in the strength of the different test portions prepared from such raw serum, and, in part, from slight variations in the receptor sensitivity (variations which would be completely inconsequential if we were working with high titer values).

Individual variations toward the weak side have already been observed. In two of the children of this family, the reactions, even in the strongest sera, were so weak that if the investigations had not been conducted in this context, they would have been considered to be insignificant, unspecific, late reactions.

Finally, it would be appropriate to mention the case of Crome* (mother M, child N) as an instructive example for a futile attempt at N_2 detection. In this case, most of the tests on the mother yielded the diagnosis M. However, three investigators observed weak N reactions (O. Thomsen even found them in two of the N sera used, thus prompting his diagnosis of MN). The mother must be MN_2 (possibly with an unusually weak N_2 -receptor). Thus Crome's own assumption that he was dealing with a latent N-receptor in the mother, receives a simple explanation.

The question, what technique should be used to cope with this source of error in MN determinations does not belong to our present

*Z. gerichtl. Med. 24: 167, 1935.

considerations. Because of a remark in Crome's work, I would like to mention that the absorption method has very little significance in this context.

For application of the MN system to questions of parenthood, the knowledge of the N_2 -receptor and the resulting uncertainty as compared to the diagnosis $M(M+ N-)$ means that certain caution must be exercised when excluding the possibility of fatherhood on the basis of blood group constellations, where the presence of an undetectable N_2 strain in one of the persons concerned would falsify the conclusion.*

How far this reservation is allowed to go depends, of course, on the frequency of error possibility. When this is found, as mentioned above, even when selected, strong sera are used, in which case the N_2 receptor generally does not escape detection, it should be considered to be the same as the frequency of the N_2 characteristic.

It is in the nature of the phenomenon that the frequency of the N_2 characteristic cannot be expressed numerically, as e. g., per thousand of the population, since it has only been observed as a family characteristic and since it is therefore possible that it might be rather frequent in certain areas, and yet be rather rare in the total population.

It would, however, seem reasonable that it must be very rare. If the N_2 characteristic would have a relatively considerable frequency, we would have found, on the basis of the simple genetic process of the MN system, other mother-child combinations which contradict the system, but the literature of 7 years reports only the one case by Crome.

On the other hand, it could be possible that there have been more cases of this kind, and that the incorrect diagnosis, prompted by the unbiological constellation could have been detected and that a weak N reaction could have been discovered by improved techniques. It would be desirable to re-investigate all cases where such a

* This is, of course, presupposing that one considers the blood group investigations with regard to the MN system, to serve as sufficient proof. If one does not deviate from pronouncements such as: "Legal fatherhood can be excluded with considerable probability" or similar ones, it is of course unnecessary to make reservations because of this rare source of error.

possibility exists; all observations of remarkably weak receptors* (see those reported in the article by Crome) would have to be analyzed and family investigations would have to be conducted.

It is in the nature of the MN system that, when such a reservation has to be made, one must distinguish between three different "exclusion types" within the system.

1. The type of mother N, child MN and reported father N, is not touched upon by the source of error in question, since the exclusions rest on the non-detection of the M characteristic.

2. In the case of a type M mother, a child of type MN and $\frac{1}{365}$ a reported father of type M, an undetectable N_2 strain can be present in one of the parents. However, N_2 individuals can have, according to the above genetic hypothesis, N_1 descendants only in connection with an N_1 partner. The fatherhood in the case of this child (which is a "usual MN", i. e., MN_1) is thus incompatible with the system, even if one parent should have a N_2 characteristic.

3. In the type of mother $\frac{M}{MN}$, a child M, and a reported father N, or a mother $\frac{M}{MN}$, a child N and a father M, i. e., in those combinations where the exclusion rests on the fact that father and child are homozygotic opposites, the fatherhood is possible if the M partner has a N_2 strain. In this area, the possibility of error could possibly play an important role.

The frequency of the three exclusion types, if we estimate the total "theoretical exclusion percent" at 18, is about 2 or 4, or 12%. If we assume the correctness of our hypothesis, this means in practice that about one-third of all exclusions according to the MN system, are not touched upon by the factor of uncertainty which is caused by the existence of the N_2 -receptor. Without the above assumption, only one-ninth is not touched upon.

In statements concerning questions of fatherhood, based on the MN system, our Institute (almost all our legal blood group investigations are done in Denmark) relies on a differentiation which corresponds

*When considering the probable frequency of weak N-receptors, one should take the possibility into account that such cases can go through the laboratory undetected, even when especially lucky in the manufacture of very strong anti-N sera.

to different degrees of certainty of the three exclusion types. This can be done rather easily, using the usual considerations concerning the application of blood group investigations in questions of legal parenthood, and the practice which has evolved from that.

According to the Danish law, it is necessary that biological proof be practically without error to be applicable to such cases. The Danish courts of law have therefore used the help for several years which the "new" blood group systems MN and A_1-A_2 can offer, although it could be said that they did not have as good foundations (which they may indeed have today) as the classical blood group system (ABO).

This was, of course, accompanied by extended application of special supplementary explanations concerned with the degree of certainty which the different fields of blood group research preferred at the respective time.

As a consequence of the slow clarification of some of the /366 viewpoints related to these questions, it was considered to be appropriate to exactly determine the formulation of the conclusions of blood group investigations, and, consequently, this Institute introduced a system of explanations in the year 1933, which formulates the significance to be accorded to the blood group investigation of each blood group combination.

The main criterion is that the incompatibility of fatherhood with a blood group system is used as proof with varying significance, depending on the blood group system in question (although in most all cases the proof is so strong that we may say the possibility of fatherhood is excluded). In the "exclusion cases" we never use the expression that a person "cannot be the father", however, we do declare that the fatherhood of the person concerned is incompatible with the ABO or MN, or the A_1A_2 system, indicating the point in question supplements the explanation, where we explain the significance of this point in the opinion of our Institute.

In this supplement, we give a short description of the blood group systems, emphasizing that in its application we are dealing with a biological method, which can never guarantee absolute certainty.

The supplement has somewhat different information about the ABO system.

The larger part of the system is said to have the greatest possible certainty: we explain that the possible occurrence of deviations from the

genetic rule can never be completely excluded and that one such case has indeed occurred. However, we stress that this possibility must be considered to be so slight that one may say that "fatherhood incompatible with the ABO system can be excluded with as great a certainty as one may ascribe to a biological method", and we add that "it must be permissible to neglect this possibility, unless very exceptional reasons, which have nothing to do with the results obtained by the blood group investigations, are in conflict with the results of the investigation."

A certain, very slight reservation is made, concerning the limited area of the ABO system stressed by O. Thomsen, where exclusion rests on the non-detection of characteristic A_2 in one partner (Hosp. tid. (Danish) 76: 169, 1933).

Since 1933, the MN system has been considered to be of similar worth as most areas of the ABO system. For this reason the wording of the conclusion, possibly containing slight reservations because /367 of the more complicated techniques, has been the same for both systems.

The A_1A_2 system has a much lower degree of certainty and incompatibility of fatherhood with this system is therefore not excluded with "biological certainty"; however, incompatibility would be considered a strong circumstance in establishing evidence against fatherhood. If the incompatibility with the system is caused by detection of the characteristic A_2B (in contrast to A_1B), we declare, on the basis of the special uncertainty in this differential diagnosis, that the fatherhood cannot be regarded quite as improbable as to consider the blood group investigation results as having decisive significance in such a case.

Since the publication of the experiences reported here, we suggest that attention be directed to this source of error appearing in the discussion of the MN system in the supplement of our Institute. (Similarly as attention was directed to the existence of the Haselhorst case in the discussions (since 1933) of the ABO system).

Concerning the significance of the incompatibility of fatherhood with the MN system, the supplement mentioned contains three different pronouncements, corresponding to the above discussion.

In the first exclusion type mentioned above, wording is the former, i. e., similar to that for the great field of the ABO system. For the second type, the wording is the same, with formal reservation at

most, i. e., the same as with the small field of the ABO system. In the case of the third type, we consider it most correct, until more experience has been collected, to declare that the possibility of error, although very slight, is yet "to be considered as not so insignificant to remain unconsidered in weighty circumstances which have nothing to do with blood group investigation, these are to be considered in deciding the correctness of the fatherhood under consideration".

This field is therefore placed in a "security class" below the ABO system and the remainder of the MN system, although above the A_1A_2 system.

As we can see, this differentiation causes no fundamental reorientation, but can be easily adapted into the system of explanation previously used, which is already familiar to the courts of law.

SUMMARY

1. One case of a mother-child combination, which, according to the genetic rules of the MN system should not be possible (mother M, child N), can be explained after detailed analysis by the fact /368 that the mother actually belongs to the MN group. with defective development of the N-receptor.
2. This weak N-receptor is a characteristic, inherited variant of the N-receptor. Its serology is described.
3. The family of the mother is investigated. The inheritance process of the characteristic (designated with N_2) seems, without unreal hypotheses, to be only explainable by the fact that it must be caused by an independent, allelomorphic gene. It thus represents an A_2 in the group character analogous to the ABO system.
4. The significance of the N_2 characteristic as a source of error in the blood group determination is discussed.

GENETIC INVESTIGATIONS OF THE BLOOD FACTOR P
IN FAMILIES AND TWINS

Peter Dahr

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GENETIC INVESTIGATIONS OF THE BLOOD FACTOR P IN FAMILIES AND TWINS*

Peter Dahr

Due to our exact knowledge of the hereditary mechanism ^{**/168} of the agglutinable blood corpuscle properties A, B, O, M and N, the determination of these properties has already become of considerable practical importance to the clarification of contested parentage. In contrast with this, the mechanism whereby A₁ and A₂ are inherited is as yet less certain so that its practical importance for the clarification of contested parentage, at least at present, is not as great as the hereditary blood properties mentioned above. This applies even less to the properties A₃ and N₂, which according to Friedenreich are attributable to the presence of special genes whose hereditary mechanism has so far been only slightly studied.

Soon after the discovery of the blood factors M and N by Landsteiner and co-workers in 1927, extensive investigations on the hereditary mechanism of these blood properties were initiated. Therefore, at present, the hereditary mechanism of these properties seems to have been ascertained. However, the factor P discovered by Landsteiner at the same time and which, according to a few studies carried out by Landsteiner at the time, is also undoubtedly hereditary, was neglected.

According to Landsteiner and Levine the factor P can be detected:

1) By means of an irregular agglutinin present in human serum, which they call "extra agglutinin I", whose activity, however, is not very high. Tests on blood corpuscles with different human sera containing extra agglutinin I carried out simultaneously showed, remarkably, that qualitatively irregular reactions ("discrepancies") occur ^{/169} occasionally. It is possible that these different reactions were caused by differences in agglutination activity between the sera which normally have low activity, in other words by a quantitative factor; in

* These studies were supported by the Association of Friends and Promoters of the University of Cologne. Miss Louise Pitts, Technical Assistant, has given valuable assistance in these investigations.

**/Numbers in the margin indicate pagination of the original foreign text.

any case, the existence of qualitative differences between the various sera or blood corpuscles were not demonstrated by absorption experiments.

2) P can be detected by means of rabbit immune serum obtained by injection of P-containing human blood corpuscles followed by purification of the serum with P-free blood corpuscles. Apart from minor differences caused by differences in titer strength, the immune sera obtained by Landsteiner and Levine showed good agreement between the reactions. The mode of reaction of the immune agglutinins also agreed very well with that of the extra agglutinin I of human serum.

3) According to Landsteiner and Levine, the detection of P is possible by means of certain animal sera (for example horse, cow, hog) after absorption of the sera with P-free blood corpuscles. The animal anti-P also agreed in general in its mode of reaction with the immune anti-P. However, irregular results were occasionally obtained during simultaneous application of several animal anti-P sera. For example, the blood corpuscles of two O humans which had reacted with extra agglutinin I, when tested with 12 different anti-P horse sera were agglutinated by only 0 of these sera. If quantitative causes (different P strengths of the blood corpuscles or different agglutinin titers of the sera) are excluded, this result means that the P of different human beings does not have to be qualitatively identical, and that the agglutinins of different origin which are active towards the complete P complex may differ qualitatively from one another, perhaps in the composition of the individual agglutinin components. Landsteiner informed me recently that he and his co-workers during the investigations carried out at that time had arrived at the conclusion that P is a "group of related agglutinogens", a conjecture which was supported by the presence of variants of the properties A and N. Accordingly, he provisionally designated the P which can be detected in human sera by means of extra agglutinin I as " P_n ", the P detectable by immune serum as " P_i ", and the P detectable by means of natural animal sera as " P_a ".

Future investigations will have to show whether or not the P of different humans and the anti-P of different animals are different. In any case, the existence of different variants of P and different agglutinin complexes would not make the detection of P less important even if one knows about this variability.

The property A is not homogeneous either, and the A_3 of Friedreich also can be detected only with certain anti-A sera (independent of the strength of the agglutinin) and not with others, so that, as I

recently discussed briefly in the Klin. Woch., No. 35, 1939, we could regard A₃ not only as a quantitatively weak A variant but also as a qualitatively special A-antigen complex. In this connection, I wish, /170 furthermore, to refer to the observation of Landsteiner and Wiener which was confirmed by me in cooperation with Lindau on the basis of experiments with monkeys, that the anti-M in different immune sera may have qualitatively different compositions. This is also confirmed by results reported by Friedenreich and Lauridsen at the 7th Scandinavian Congress of Pathologists in 1938.

The impetus for the investigations reported at the time by these two investigators was a remarkable observation in a MN child, whose M could not be detected by means of a certain, otherwise quite active anti-M immune serum. The conception that the M of the child and the anti-M of the aberrant immune serum deviated qualitatively from other M properties or from other anti-M immune sera could be confirmed by means of absorption experiments. Initially, the M of the child was regarded as "defective". A study of the blood corpuscles of the child by means of more than 30 different immune sera obtained from other laboratories (ours among them) showed that the majority of the sera reacted with the blood corpuscles whereas 5 M sera gave no reaction with the customary technique. Titration of the positively reacting sera against the blood corpuscles of the child in comparison with normal MN blood corpuscles showed that more than half of the sera reacted more weakly with the blood corpuscles of the child than with the control blood corpuscles; about 1/4 reacted equally strong with both types of blood corpuscles, and the remaining fourth agglutinated the blood corpuscles of the child more strongly than the control blood corpuscles. On the basis of these results, the M of the child could no longer be regarded as a "defective" M; as it obviously possessed M components not present in other M-containing blood corpuscles, it could rather be regarded as an "unusual specialized" M. Investigations with a number of family members showed that this "unusual" M was apparently hereditary.

Similarly, Dombrowsky observed that the N of an MN human could not be detected with a certain, otherwise quite active N serum. Here, too, we may assume that a qualitatively deviating N is present in MN humans and that an anti-N of a special composition is present in the serum used.

The importance of the M or N detection is not reduced by the reported results and the conclusions drawn from these results. In the same way, the possible importance of P detection in the sense of the utilization of M and N determination is not reduced by the possibility

that the P agglutinin in different humans and the anti-P in different sera might have different complex compositions.

When, in 1937, I wanted to start investigations on the factor /171 P in Cologne, I wrote to Prof. Landsteiner in New York and asked him to send me some anti-P serum for preliminary studies. At that time, Landsteiner informed me that he had not had any anti-P serum available for quite some time. In Europe, an anti-P could not be obtained either. Due to a coincidence, in the beginning of 1939, I was able to find anti-P in natural hog serum.

For the purpose of isolating anti-O agglutinin, I absorbed a certain hog serum (No. 176) with certain A₁B blood corpuscles. The serum thus obtained showed the following effect towards the blood corpuscles used for the absorption and a number of different O blood corpuscles:

HOG SERUM 176, ABSORBED WITH A₁B TITRATED AGAINST BLOOD CORPUSCLES

Titer:	1	2	4	8	16	32	64	128	256
A ₁ BN (Tü.)	-	-	-	-	-	-	-	-	-
OMN (Sauer)	+++	+++	++	+	+	-	-	-	-
OMN (Girt.)	+++	+++	+++	+++	+++	++	++	++	+
OM (Dell.)	+++	+++	++	+	+	-	-	-	-
ON (Kiedr.)	+++	+++	+++	+++	+++	++	++	++	+

I thought at first that I was dealing with O blood corpuscles which differed in degree of agglutinability and which could be distinguished by means of this certain anti-O serum. It was found, however, that in spite of strong absorption with A₁B the hog serum still agglutinated many A and B blood corpuscles, in part to a titer of up to 256; accordingly, the activity towards the strongly agglutinated O blood corpuscles was apparently no anti-O activity. This was confirmed by the fact that after absorption with the weakly agglutinated O blood corpuscles (Sauer) this serum no longer agglutinated O blood corpuscles, whereas it still agglutinated the O blood corpuscles Girt. and Dell. at the same strength. Thus, the hog serum absorbed with A₁BN (Tü) and OMN (Sauer) had to contain another agglutinin which was active towards an agglutinable property which was not present in the blood corpuscles used for absorption, but was present in many other blood corpuscles of all groups. A

number of preliminary tests with twins and a number of families made it probable that this was a hereditary blood corpuscle property. My conjecture that the detected agglutinin was identical with the factor P was confirmed by Prof. Landsteiner who was kind enough to test 172 a sample of my serum on persons which he knew did or did not possess P. I then started genetic studies on P which involved 300 pairs of twins and 112 families with 434 children, the results of which are discussed in this publication. *

First, I will briefly discuss the sera used and the technique employed. For the P determination, only animal sera were used. As I had P-free AB and O blood corpuscles available right from the start, I began immediately with the preparation of new anti-P sera by absorption of other animal sera. The results obtained hereby will also be presented here.

According to Landsteiner and Levine, one finds anti-P fairly often in the serum of rabbits, hogs, cats and cows but particularly frequently in horse sera.

For the purpose of preparing new animal anti-P agglutinins, I have so far investigated 198 cow sera, 292 hog sera and 186 sheep sera which were obtained from the slaughter house in Cologne in quantities of 30 to 40 in 750 cc wide-mouth flasks. Horse sera could only be obtained rarely and in small quantity so that we decided initially not to study them.

After inactivation, the sera were diluted 1:4. In order to save absorption blood, we used only small quantities of sera or of dilutions: 1 drop of serum + 3 drops of NaCl solution for the preliminary absorptions; in order to remove the undesired agglutinins we added 1 drop of washed, densely centrifuged blood corpuscle sediment A₁BN and OMN to each of these mixtures; both types of blood corpuscles were P-free. Then, we carried out the absorption at +5° with occasional shaking; next, the blood corpuscles were removed by centrifugation and the serum dilutions were tested against the P-free blood corpuscles used for absorption and against P-containing blood corpuscles. Those sera which still agglutinated the P-free and also the P-containing blood corpuscles were further absorbed with a suitable quantity of P-free blood corpuscles. The sera which no longer agglutinated the P-free blood

*A short preliminary communication on these investigations has been published in Klin. Woch., No. 23, 1939.

corpuscles but still showed activity towards the P-containing ones, were selected and tested further. The sera which were completely inactive towards both types of blood corpuscles (P+, P-) after absorption were eliminated right away. By absorption of serum dilutions /173 1:4 we eliminated right from the start the poorly active sera with a titer lower than 4. The number of sera found and their titer values (tested against P-containing A₂MN (Trau.) blood corpuscles) are listed in the table below.

No.	Type of animal	Titer of the crude serum
176	hog	256
5	"	8
21	"	8
34	"	8
82	"	8
84	"	8
92	"	8
99	"	4
121	"	8
188	"	8
130	sheep	16

Compared with the hog serum I first found (176), the titers of the anti-P sera found later are relatively low. Landsteiner informed me that the titer of serum 176 was higher than that of the animal sera found by him at the time.

When we initially began to use a new serum it was first compared over a prolonged period with the serum used previously. Occasional minor discrepancies in the reaction could always be explained on the basis of deficient absorption of the undesired agglutinins and could accordingly be eliminated every time. In using my animal sera I have so far never made any observations which pointed to qualitative differences between the individual sera. In this connection, the always-similar results obtained with the identical twins studied by me are of special importance. Among the 198 cow sera tested not one was found to be useful.

Hog sera were found to be the most productive and, therefore, I would recommend hog sera as the most suitable for finding animal anti-P agglutinin even though, according to Landsteiner and Levine,

anti-P is also frequently found in horse sera. The latter are certainly less easy to obtain.

I have not included (for example, for comparative studies) /174 the "extra agglutinin I" of Landsteiner (the anti-P agglutinin present in human sera) as I could not find a suitable serum.

My co-worker Huntgeburth is at present engaged in the determination of the productivity of human sera in regard to extra agglutinin I. These systematic studies involve a very large number of sera and will be reported elsewhere. So far, he has not found a useful one among 1000 human sera. This result is not in agreement with the data of Landsteiner and Levine who report that human sera containing extra agglutinin I are not rare; however, more detailed numerical information is lacking so that the results may just appear to be in disagreement. In addition to these studies I would recommend a search for anti-P in animal sera especially in those cases where numerous human sera can be studied; the titer of animal sera is higher than that of human sera.

In my investigations, I have not yet been able to use immune anti-P agglutinin since up to the present I have not been able to produce it. Immunization experiments with the usual technique have so far been unsuccessful with 10 rabbits. By subsequent use of the procedure recommended by Hilgermann, in 1938, which is supposed to be 100% successful for M and N immunization, I have not achieved any positive results.

In producing immune anti-P, Landsteiner and Levine used the following technique: at intervals of 6 days, the animals received increasing quantities of blood corpuscle sediments, 0.1 to 0.4 cc suspended in 1 cc of NaCl solution. After a rest period of 8 days, a second similar immunization series is started which after a similar period is sometimes repeated several times in case intermittent tests for anti-P formation give negative results.

The P determination was carried out using the microscopic slide method by mixing of a drop of serum with a drop of a 3 to 5% suspension of the blood corpuscles to be studied. Readings are taken after 15 minutes at room temperature. The study involved 300 pairs of twins and 112 families with 434 children.

The addresses of the twins and the written consent of the /175 parents to the investigation of the twins were obtained through the Department of Health of the city of Cologne for which I am indebted to its director, Prof. Dr. Coerper. Some of the addresses of the twins were

obtained on request from the heads of the Cologne high schools. Numerous other serological tests were carried out with the twins which will be reported in another publication. I carried out the similarity diagnosis myself after I had become familiar with this procedure during a stay at the Kaiser Wilhelm Institute for Anthropology, Human Genetics and Eugenics, in Berlin-Dahlem; I am greatly indebted to the director of the Institute, Prof. Eugen Fischer.

In making the similarity diagnosis, we determined the blood groups, subgroups, factors and a series of bodily characteristics such as color of hair, form of hair, hair insertion in the neck, eyebrows, fold in the upper eyelid, eyelashes, color of the eyes, structure of the iris, color of the skin, anatomical details of the nose, the ears and the oral region and to a certain extent the fingernail cuticles as well. With many twins of the same sex which looked alike and were the same in regard to groups and factors, the diagnosis "identical" or "fraternal" initially presented difficulties; these pairs were seen again later when the diagnosis was easier to make because of a little more practice. In many doubtful cases, the different result of the group, subgroup or factor determination led to the unequivocal diagnosis of "fraternal".

The P determination was carried out independently of the similarity diagnosis so that involuntary influences can be excluded as sources of error. According to the similarity diagnosis, 123 pairs of the 300 pairs of twins were identical, and 177 pairs were fraternal.

The results of the P determination are shown in the following table:

EZ = 123 pairs, among which	P : P = 103 pairs (concordance)
	p : p = 20 pairs (concordance)
	P : p = 0 pairs (discordance)
ZZ = 177 pairs, among which	P : P = 122 pairs (concordance) / 176
	p : p = 15 pairs (concordance)
	P : p = 40 pairs (discordance)

(The presence of P is indicated by P and its absence is indicated by p; P : P means P present in both twins, p : p means absence of P in both twins, etc.).

The fact that the presence or absence of P is not discordant in any identical pair of twins, whereas there is a discordance with respect to P in 40 of the 177 fraternal twins favors the heritability of P in agreement with earlier observations by Landsteiner.

In their initial studies on P, Landsteiner and Levine had already found that the strength of the P property is different in different human beings. In order to clarify the question regarding the hereditability of the P strength, we titrated the blood corpuscles against dilutions of the highly active hog serum 176 for a number of twins. These determinations of the agglutinability titer were continued as long as hog serum 176 was available. The anti-P sera found later seemed to me to be too poorly active for comparative titer tests in order to obtain differences which could be clearly evaluated. The titrations were carried out by means of the microscopic slide technique.

Readings were taken after 15 minutes at room temperature. The results of the P titer determination in 123 P positive pairs of twins are shown in the following table:

EZ = 56 pairs, among which P titer was the same = 51	
among which P titer was different	
	1 stage = 5
	2 and more stages = 0
ZZ = 67 pairs, among which P titer was the same = 38	
among which P titer was different	
	1 stage = 19
	2 and more stages = 10

It is true that single stage titer differences do not prove that the titers are actually different. Accordingly, a clear difference in P strength exists only in the fraternal pairs of twins, and the fact that such a clear difference is not present in identical pairs of twins favors the hereditability of the P strength. Thus, the situation appears to be similar to that found with the A property which, as is known, occurs also in different strengths, whereby the strong A, A₁, and the weak A, A₂, appear to be based on a special gene. /177

As already mentioned above, the twins studied involved persons of grade school and high school age up to about 20 years of age. In order to clarify the question whether the P property can be detected in still younger persons and perhaps even in neonati, I have begun to study umbilical blood samples for P in cooperation with Wiesener (Gynecology Clinic of the University of Cologne). In 194 blood samples tested so far, P could be detected 166 times and could not be detected in 28 cases. If the error introduced by the small number of tests is considered, this ratio corresponds approximately to the numbers found in adults, which we will discuss below.

For the question of a special hereditary mechanism of the P strength, like in the case of A_1 and A_2 , and a possible practical utilization of the inheritance of the P strength for resolving cases of contested parentage, the question of a titer change in P strength is decisive. This question, too, is being studied in cooperation with Wiesener. Hereby, the neonati tested for P are again repeatedly tested later on, whereby it is determined whether the agglutinability found initially becomes stronger or not during aging and, furthermore, whereby we observe whether initially negative blood samples become positive or remain negative with increasing age. The investigations are still in progress, and the results will be published later elsewhere.

Studies on Families

The first and only family studies involving P were carried out by Landsteiner and Levine and were published in 1930. It is remarkable that even in America, where, as a result of the first studies by Landsteiner and co-workers, the basis had been provided for further studies, no further genetic studies have been carried out. Landsteiner informed me that it was very difficult for him to obtain material for genetic studies; besides, in his opinion, the interest in human serology is not very high in America.

At the time, Landsteiner in cooperation with Levine tested 178 59 white families and 44 Negro families, a total of 103 families, with human anti-P agglutinin (extra agglutinin I). Hereby, they distinguish between the following reactions strengths: 1) $+$ \pm to \pm ; 2) "trace"; 3) "very small trace" or minus; they concluded from their results that P is undoubtedly hereditary. It was remarkable that the stronger reactions were more frequent in Negroes than in whites, in which the P frequency was also lower. From the observation that parents who both showed reaction strength 3, produced children with reaction strength 1, Landsteiner and Levine concluded at the time that the agglutinable property P "is not a single mendelian factor like the iso-agglutinogens A and B", but that P "depends upon multiple genetic factors". However, I believe that the determination of the agglutinability strength carried out with the weakly active extra agglutinin I can not be regarded as adequate for the formulation of a strength classification of P; it is for this reason that I have decided not to carry out titer determinations with my animal anti-P sera which had a titer of at least 8, because clear differences in P strength could not have been detected anyway by means of these not very active agglutinins. Landsteiner himself considers the agglutinin used at the time for the family studies so weak that it is certain that a number of P-positive samples were not

recognized as such, since in later investigations with other persons, the P property was found in a higher percentage of cases with immune serum (with higher titer!) than in tests of the families with extra agglutinin I (private communication by letter). Accordingly, the strength classifications of P and their distribution over parents and children arrived at by Landsteiner and Levine in family studies with the weakly active agglutinin can not be used to draw conclusions regarding the heredity of P and especially the heredity of the strength of the P property.

My own family studies involved 112 families with 434 children. The blood samples were collected in Rousscher solution and after 1 "washing" the blood corpuscles were suspended in 3 to 5% physiological salt solution and then tested. We used the microscopic slide technique; readings were taken after 15 minutes at room temperature.

I attempted to answer 2 questions: 1) heredity of the P property as such and 2) the question regarding a possible unusual heredity /179 mechanism of the P properties with different strengths.

The results of the agglutinability determinations in the twins indicate that the strength of the P property appears to be genetically controlled. However, the question remains whether, similar to A₁ and A₂, we should assume special genes for the differently-active B properties and how these possible genes behave with respect to each other in regard to being dominant or recessive.

In order to answer the first question, simple determination of P in the other members of the family is sufficient; solution of the second problem would require agglutinability determinations of the P positive blood corpuscles. Since I had highly active serum 176 available in limited quantities only and since I did not want to use the animal anti-P sera obtained later in view of their low titer for these titrations, the agglutinability determinations were carried out only with 50 families.

In the families studied, we determined at the same time the blood groups, subgroups and MN factors. I have designated the presence of P as "P", and its absence as "p".

As regards the simple P determination we found that P was always present in children only when it could be detected in at least one of the parents. Accordingly, in marriages p:p we never found P children. In contrast with this, we found, in P:P marriages, P as well as p children and the same was true for P:p marriages.

The observation that P can be detected only in children when it is present in at least one of the parents contradicts to a certain extent the results obtained by Landsteiner and Levine in family studies, but this contradiction is only apparent. They observed, in cases where both parents reacted according to the reaction classification 3 (that is, "very small trace to minus"), children of reaction classification 1 ($+$ to $+$) which also led them to assume a complicated hereditary mechanism of P. This observation is only seemingly in contradiction with my results because Landsteiner admits himself that reaction classification 3 certainly includes persons possessing P whose P was not /180 detected only because weak serum was used.

My family results are compatible with the assumption that the presence of P (the question of a special hereditary mechanism of the differently strong P properties is not touched upon here) is based on a pair of genes Pp, whereby P is the dominant gene for the presence and p is the recessive gene for the absence of P.

Accordingly, there exists the following genetic pictures and visible pictures:

Genetic picture	Visible picture
PP	P
Pp	P
pp	P

The results of the family studies tabulated below, include the genetic pictures to be assumed, if this hereditary mechanism is assumed, from which the phenotype can then be obtained directly. Accordingly, PP and Pp mean presence and pp means absence of P. P means presence of P for an unknown heredity picture.

Accordingly, the following different marriages give the following different children:

Parents:	P:P = 73	P:P = 30	p:p = 9
Children:	246(P); 28(p)	77(P); 39(p)	1(P); 43(p)

The preceding table shows that the results obtained with all families are compatible with the assumption of the hereditary mechanism described above, except for the results obtained with family no. 45. Here, the following result was obtained:

	Factor types	Genetic picture
Father	A ₂ MN p	pp
Mother	A ₂ MN p	pp
1. Child, 12 years old	O MN p	pp
2. Child, 7 years old	O MN p	Pp
3. Child, 6 years old	A ₂ MN p	pp
4. Child, 3 years old	O N p	pp

The results of the P determination in child no. 2 is not compatible with the hereditary mechanism assumed for P because the /183 parents who are both p-negative can pass on only recessive p genes. The result was at first not retested in order not to arouse suspicion in the family. Later on we decided not to carry out a retest because I had found out that the second child was considered illegitimate, which is admitted by the mother. However, at a later suitable time, we plan to carry out a genetic-biological study with the family in order to render the illegitimacy probable, also objectively, or to make it certain. In any case, the P result obtained with child no. 2 of family no. 45 /184 does not contradict the hereditary mechanism of P which I have proposed, because the child is obviously illegitimate.

On the basis of my family results obtained so far I have considered it justifiable to render an expert opinion in an alimony case based on the P test in the persons concerned, stating that the defendant was probably not the father of the plaintiff (child). The mother of the child, the plaintiff and the defendant all possess the blood formula OM; accordingly, the defendant's parentage could not be excluded on the basis of the group and the MN factor determination. The P study with 3 different anti-P sera gave the following results: mother p (pp)*, defendant p (pp), and plaintiff (child) P (Pp). On the basis of the court records it could be assumed that the mother of the child had had relations with other men within the legal conception period which, of course, was not decided by expert opinion.

As regards the different agglutinability of the P-containing blood corpuscles of different humans, the conditions with the P property appear to be similar to those of the A property. If the P properties of different strengths should be based on the presence of different genes,

*Assumed hereditary pictures given in parentheses.

No.	Father	Mother	Children				/181
1.	O MN Pp	O N Pp	O MN P	O N P	O MN P	O N P	
2.	A ₁ N Pp	A ₁ MN Pp	A ₁ MN pp	A ₁ MN P			
3.	O N Pp	A ₁ N Pp	A ₁ N pp O N p	A ₁ N pp	O N P	A ₁ N P	
4.	A ₁ B M P	B MN P	A ₁ B MN P	A ₁ B MN P	A ₁ MN P		
5.	A ₁ N Pp	O M Pp	A ₁ MN pp O MN P	O MN P	A ₁ MN P	A ₁ MN P	
6.	O N P	O MN pp	O N Pp	O MN Pp	O N Pp	O MN Pp	
7.	B MN P	A ₁ B MN pp	B MN Pp	B N Pp	B MN Pp	B MN Pp	
8.	A ₁ B MN P	B MN P	A ₁ B MN P				
9.	O N pp	O MN Pp	O MN pp O MN pp O N pp	O MN Pp O MN Pp	O MN pp O MN Pp	O N pp O N Pp	
10.	A ₁ MN Pp	O MN Pp	O M P A ₁ MN P	O MN P A ₁ M P	O MN P O MN pp	O N pp	
11.	O MN Pp	O MN Pp	O MN Pp O M pp	O MN Pp	O M pp	O MN Pp	
12.	B MN P	A ₁ M P	A ₁ MN P A ₁ MN P	B MN P	B M P	B M P	
13.	O MN P	O N P	O N P	O N P	O MN P	O MN P	
14.	O MN pp	O MN pp	O MN pp O MN pp	O MN pp	O MN pp	O MN pp	
15.	B MN pp	O N P	B MN Pp B N Pp	O N Pp O N Pp	B N Pp O N Pp	B MN Pp	
16.	O MN P	A ₁ MN P	O MN P	O MN P	A ₁ MN P	O MN P	
17.	O MN P	O MN P	O MN P				
18.	A ₁ MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P		
19.	O N P	A ₂ MN P	A ₂ MN P	O MN P	O MN P		

No.	Father	Mother	Children			
20.	A ₁ MN P	B MN P	B MN P	B MN P	A ₁ MN P	A ₁ B MN P
21.	A ₁ MN P	A ₁ N P	A ₁ MN P	A ₁ MN P		
22.	O M pp	A ₁ MN P	A ₁ M Pp			
23.	A ₁ MN pp	A ₂ M P	A ₁ M Pp	A ₁ M Pp		
24.	A ₁ MN Pp	A ₁ N pp	A ₁ N Pp	A ₁ N Pp	O N pp	A ₁ N Pp
25.	O MN P	O MN P	O M P	O MN P	O MN P	
26.	O N P	A ₁ MN P	A ₁ MN P	O MN P	O MN P	
27.	O MN P	O N P	O MN P O N P	O N P O MN P	O MN P O N P	O MN P
28.	O A ₁ N Pp	O M pp	O MN Pp	O MN pp	O MN pp	
29.	O MN pp	A ₁ MN pp	A ₁ M pp	A ₁ MN pp	A ₁ MN pp	A ₁ N pp
30.	O N Fp	B MN Pp	O MN P	B N pp	B MN P	
31.	A ₁ MN Pp	A ₁ MN Pp	A ₂ MN P	A ₁ MN pp		
32.	A ₁ MN P	A ₁ MN P	A ₂ MN P A ₁ MN P	A ₂ MN P A ₁ N P	A ₁ N P	A ₁ N P
33.	A ₁ MN P	O N P	A ₁ MN P	A ₁ MN P		
34.	O N pp	A ₁ N P	O N Pp	O N Pp		
35.	A ₁ MN pp	A ₁ MN Fp	A ₁ N Pp	A ₁ M Pp	A ₁ N pp	
36.	A ₁ MN pp	A ₁ MN Pp	A ₁ MN pp A ₁ N Pp	A ₁ MN Pp A ₁ MN pp	A ₁ N Pp A ₁ MN Pp	A ₁ MN pp
37.	A ₁ MN pp	B MN P	A ₁ B MN Pp	A ₁ B MN Pp		
38.	A ₁ MN P	O MN P	A ₁ MN P A ₁ MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P
39.	B N pp	A ₁ B N pp	B N pp	A ₁ B N pp		
40.	O N P	A ₁ MN P	A ₁ N P	O N P		

No.	Father	Mother	Children			
41.	O MN pp	A ₁ MN Pp	O MN Pp	A ₁ MN pp	A ₁ MN pp	O MN Pp
42.	O MN P	A ₁ MN P	A ₁ MN P A ₁ MN P	A ₁ MN P	A ₁ MN P	O MN P
43.	B N P	A ₁ MN P	A ₁ MN P A ₁ MN P	A ₁ B MN P	A ₁ MN P	A ₂ MN P / 182
44.	A ₁ B MN P	A ₁ MN P	A ₁ M P A ₁ M P	A ₁ MN P	A ₁ M P	A ₁ MN P
45.	A ₂ MN pp	A ₂ MN pp	O MN pp	O MN Pp(!)	A ₂ MN pp	O N pp
46.	O MN P	O MN P	O MN P	O MN P		
47.	A ₁ M P	O MN P	O MN P	O M P	A ₁ MN P	
48.	O MN P	A ₁ B MN P	B M P A ₁ MN P	B N P	B N P	A ₁ MN P
49.	B M pp	O M pp	B M pp	B M pp	O M pp	O M pp
50.	B M P	A ₁ N P	O MN P	A ₁ MN P	B MN P	A ₁ MN P
51.	O MN pp	A ₁ B MN P	A ₁ M Pp	B MN Pp		
52.	O MN Pp	O MN Pp	O MN P	O MN pp	O M P	
53.	A ₁ N P	O M P	A ₁ MN P A ₁ MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P
54.	A ₁ MN P	A ₁ MN P	A ₁ MN P O M P	A ₁ M P A ₁ MN P	A ₁ M P A ₁ MN P	A ₁ M P
55.	O MN P	O M P	O MN P			
56.	O M P	O MN P	O MN P	O MN P		
57.	A ₁ MN P	A ₂ MN P	A ₁ MN P	A ₁ MN P		
58.	A ₁ MN P	O MN P	O MN P	A ₁ MN P		
59.	A ₁ MN Pp	A ₁ MN pp	O MN Pp	O MN Pp	A ₁ MN Pp	O MN pp
60.	O M P	A ₁ MN P	A ₁ M P	O M P	O MN P	

No.	Father	Mother	Children			
61.	O MN Pp	A ₁ M Pp	A ₁ M pp	A ₁ M Pp	A ₁ MN pp	
62.	A ₂ M Pp	O N Pp	O MN P O MN P A ₂ MN pp	O MN P O MN pp	O MN P O MN P	O MN P A ₂ MN pp
63.	A ₁ N Pp	O MN pp	A ₁ N Pp A ₁ N Pp	A ₁ MN pp O MN Pp	A ₁ MN Pp	A ₁ MN Fp
64.	A ₂ MN Pp	A ₂ MN Pp	A ₂ MN P	O MN P	A ₂ N pp	
65.	O MN pp	O MN Pp	O MN pp	O MN pp	O MN Pp	
66.	O MN Pp	O MN pp	O MN pp O MN Pp	O MN pp	O MN pp	O M pp
67.	B MN P	B MN P	B MN P O MN P	O MN P B MN P	B MN P O MN P	B MN P
68.	A ₁ MN Pp	A ₁ MN pp	A ₂ MN pp	A ₁ MN Pp	A ₁ MN Pp	
69.	O MN Pp	A ₁ N Pp	A ₁ MN P	A ₁ MN pp	A ₁ N Pp	
70.	O MN pp	A ₁ MN pp	O MN pp O MN pp	O MN pp O MN pp	A ₁ MN pp	O MN pp
71.	O MN P	A ₂ B MN P	A ₂ MN P A ₂ MN P	B MN P	A ₂ MN P	A ₂ MN P
72.	A ₁ MN Pp	A ₁ B MN Pp	A ₁ MN P	A ₁ B MN pp	A ₁ MN P	A ₁ B MN P
73.	A ₁ MN pp	O MN pp	A ₁ MN pp O MN pp	O MN pp A ₁ MN pp	A ₁ MN pp A ₁ MN pp	O MN pp
74.	O MN P	A ₁ M P	O MN P	O M P	A ₁ M P	
75.	O MN Pp	O MN Pp	O M P	O MN pp	O MN pp	O MN pp
76.	A ₁ M Pp	A ₁ MN Pp	O MN P O MN pp	A ₁ MN P A ₁ MN P	A ₁ MN P A ₁ MN P	O M P
77.	A ₁ B M Pp	O MN Pp	B MN P B MN P	A ₁ MN pp	B MN pp	A ₁ MN P
78.	A ₁ M Pp	A ₁ MN pp	A ₁ MN pp A ₁ MN Pp	A ₁ MN pp	A ₁ MN Pp	A ₁ MN pp

No.	Father	Mother	Children			
79.	A ₁ MN pp	B M pp	A ₁ B MN pp A ₁ B MN pp	A ₁ MN pp B MN pp	B MN pp B MN pp	B MN pp
80.	O MN Pp	B M pp	B MN Pp	B MN pp		
81.	A ₁ MN P	A ₂ MN P	O N P	O MN P	O MN P	
82.	O MN Pp	O MN pp	O MN pp	O MN Pp		/183
83.	A ₁ MN pp	O MN pp	O N pp A ₁ MN pp	A ₁ MN pp	A ₁ N pp	O MN pp
84.	A ₁ MN P	A ₁ MN P	A ₁ N P			
85.	A ₁ N P	A ₁ N P	A ₁ N P A ₁ N P	A ₁ N P	A ₁ N P	A ₁ N P
86.	O MN Pp	O MN Pp	O MN pp	O MN pp	O MN P	
87.	O MN pp	A ₁ N Pp	A ₁ MN pp	A ₁ N pp	O N Pp	O N Pp
88.	O MN P	A ₁ N P	A ₁ MN P	A ₁ MN P	A ₁ N P	
89.	O M pp	O N P	O MN Pp	O MN Pp	O MN Pp	O MN Pp
90.	B MN Pp	O MN Pp	B MN P B MN pp	B MN P	B MN P	B M P
91.	O MN P	B N P	B MN P	O MN P	B MN P	B MN P
92.	A ₁ MN P	O MN P	O MN P O MN P	O MN P O MN P	A ₁ MN P	O MN P
93.	O MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P	
94.	O MN P	A ₂ MN P	O MN P	A ₂ M P		
95.	B MN P	B MN P	B MN P B M P	O MN P	B M P	B M P
96.	A ₁ MN Pp	O MN Pp	A ₂ MN P A ₂ M P	A ₁ N P A ₂ N P	A ₁ M pp	A ₂ M P
97.	A ₂ MN Pp	O N pp	A ₂ N pp	O MN pp	A ₂ N pp	A ₁ MN Pp

No.	Father	Mother	Children			
98.	A ₁ MN P	O MN P	A ₁ MN P	A ₁ MN P		
99.	A ₁ MN P	O N P	A ₁ MN P O N P	O MN P	O N P	A ₁ N P
100.	O MN P	A ₁ N P	A ₁ MN P	A ₁ MN P		
101.	O M P	A ₁ MN pp	A ₁ MN Pp	O MN Pp	A ₁ MN Pp	
102.	A ₁ MN P	O MN P	O MN P	O MN P	O MN P	O MN P
103.	B MN P	O N P	B N P	O N P		
104.	A ₁ MN P	A ₂ MN P	A ₂ MN P	A ₂ MN P		
105.	O N Pp	A ₁ MN pp	A ₁ MN pp	A ₁ MN pp		
106.	O MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P	A ₁ MN P	
107.	O MN P _o	O N pp	O MN Pp O MN pp	O N pp O N Pp	O N Pp O N Pp	O N Pp O MN Pp
108.	O MN P	O MN P	O MN P	O MN P		
109.	A ₁ N P	A ₁ MN pp	A ₁ N Pp	A ₁ N Pp		
110.	O MN P	O M P	O MN P	C MN P		
111.	A ₁ MN P	O MN P	A ₁ MN P	A ₁ N P		
1	O MN P	A ₁ MN P	A ₁ N P A ₁ MN P	A ₁ M P A ₁ MN F	A ₁ N P A ₁ N P	A ₁ N P A ₁ MN P

similar to A₁, A₂ and A₃, a separation of the various P "subgroups" by means of simple agglutinability determination, in my opinion, could not be carried out anymore unambiguously than determination of the A subgroups. In my experience, this can, in many cases, not be irrefutably done by simple titration of the blood corpuscles with anti-A serum. Accordingly, in determining possible P "subgroups", the use of certain qualitative methods of the type we have available for the A subgroup determination in a₁ serum would be useful. It may be that the differences could be determined unequivocally by means of absorption methods as recommended by Friedenreich and Worsaae for the determination of the A subgroups, which, however, are too cumbersome from a practical standpoint. A condition for using methods also by means of which qualitative differences between the various P forms could be detected, would,

of course, be that there should exist certain qualitative differences between these forms, even if small.

Therefore, I can not draw any conclusions in regard to a /185 possible subdivision of the P property on the basis of the titer determinations in the families studied here, the number of which was limited. This whole question should be studied more intensively than has been possible so far, by new family studies after successful isolation of an immune serum or a highly active animal serum. I would like to mention here just one single observation, namely, that we never observed a strong P titer (that is, strong P) in children when in the parents only a weak P was present. Conversely, in the marriages P strong : P strong, children with both P strong and P weak were found. In the marriages P negative : P strong, we found both P strong and P weak children. Although I do not definitely commit myself, it could be concluded from these results that there might be a special gene for P strong which would be dominant with respect to a possible special gene for P weak. The titration results obtained with a number of families would agree very well with this opinion:

	Blood formula	P titer	Assumed genetic picture
<u>Family 15:</u>			
Father	B MN p	0	pp
Mother	O N P	64	P strong/P weak
1. Child	B MN P	8	P weak/p
2. Child	O N P	64	P strong/p
3. Child	B N P	8	P weak/p
4. Child	B MN P	8	P weak/p
5. Child	B N P	8	P weak/p
6. Child) = EZ	O N P	64	P strong/p
7. Child)	O N P	64	P strong/p
<u>Family 17:</u>			
Father	O MN P	64	P strong/P weak
Mother	A ₁ MN P	4	P weak/P weak
1. Child)	O MN P	4	P weak/P weak
2. Child) = EZ	O MN P	4	P weak/P weak
3. Child	A ₁ MN P	64	P strong/P weak
4. Child	O MN P	4	P weak/P weak

	Blood formula	P titer	Assumed genetic picture
<u>Family 21:</u>			
Father	A ₁ MN P	4	P weak/P weak
Mother	A ₁ MN P	128	P strong/P weak
1. Child	A ₁ MN P	128	P strong/P weak
2. Child	A ₁ MN P	4	P weak/P weak
3. Child	A ₁ MN P	4	P weak/P weak
<u>Family 29:</u>			
Father	O MN P	64	P strong/P weak
Mother	O MN P	4	P weak/P weak
1. Child	O M P	64	P strong/P weak
2. Child	O MN P	4	P weak/P weak
3. Child	O MN P	64	P strong/P weak

In addition to the reaction pictures presented here, there /186 were families in which all P carriers possessed a weak P. The titer boundaries were not delineated as clearly in all families as they were in the families discussed above. For this reason, and also because the number of families studied by means of titration of the blood corpuscles is still very small, I can not as yet draw any definite conclusions from the results in regard to a hereditary mechanism of the titer strength; I can only state that a strong P apparently occurs in children only when it is present in at least one of the parents.

Finally, I would like to present some results on the P frequency in Cologne. The number of persons studied so far for P is 1560. Among these 1293 were P positive, which is 82.8%, and 267 (17.2%) were P negative.

In an earlier publication in Klin. Woch., No. 23, 1939, I have compared all of the data on the P determination in Cologne with the data obtained by Landsteiner and Levine in their family studies in North America. At that time, these two authors had found the factor P occurring more frequently in Negroes than in whites. My data determined for Cologne at the time (obtained with 362 persons: P⁺ = 80.9%; P⁻ = 19.1%) were the same as those found in Negroes, whereas whites possessed P less frequently. The Landsteiner data which I cited at the time were:

Whites: $P^+ = 44.2\%$; $P^- = 55.8\%$

Negroes: $P^+ = 71.1\%$; $P^- = 28.9\%$

In a letter, Landsteiner pointed out to me that the P-positive numbers obtained in his family studies did not allow any conclusion on the actual absolute frequency of P and, therefore, could not be compared with my Cologne data. In the study with the weakly active /187 extra agglutinin I it is certain that a number of P-positive samples were not picked up. At most, the data given could be regarded as an expression of the relatively different frequency of P in whites and Negroes.

Actually, the results obtained by Landsteiner and Levine with more highly active immune sera in 265 whites and 267 Negroes showed different percentages:

Whites: $P^+ = 81.9\%$; $P^- = 18.1\%$

Negroes: $P^+ = 97.8\%$; $P^- = 2.2\%$

Thus, the values found with American whites closely approached those of the values found by me in Cologne. In contrast with this, the American Negroes are virtually purebred P. Perhaps, all anthropologically purebred Negroes are completely purebred P and the fact that the occurrence of P in American Negroes is not completely 100% would then be caused by slight mixing with white blood. In the purebred original (aborigines) inhabitants of America it has also been found that they are purebred O. In any case, from an anthropological viewpoint, a study of other different populations and races would be interesting.

My results can be summarized as follows:

In investigations involving 300 pairs of twins (among which were 123 identical and 177 fraternal), all identical pairs showed no discordance in regard to the agglutinable blood property P, whereas in 40 of 177 fraternal twins a discordance was present. In agreement with earlier observations by Landsteiner and co-workers, these results favor the heritability of the P property.

In agglutination tests with 123 P-positive pairs of twins (56 identical, 67 fraternal), the identical pairs showed no discordance in regard to the P strength; however, in 10 of 67 fraternal pairs a discordance in regard to the P strength was present. These results indicate that not only the P property, as such, but also the strength of the P property is hereditary.

In studies on 120 families with 434 children, it was found that P was always present in children only when it was detectable in at 188 least one of the parents; accordingly, in marriages p:p there were always only children without P, with the exception of one child which was found to be illegitimate.

Results of the family studies agree with the assumption that the hereditability of P is based on a simple mendelian pair of genes, Pp, whereby P is the dominant gene for the presence, and p the recessive gene for the absence of P.

A certain special hereditary mechanism of the various strengths of the P properties could not yet be recognized due to the relatively small number of families studied in this respect. It seems, however, that marriages P weak : P weak do not result in children with strong P.

The factor P can also be detected in neonati. It could not yet be determined whether during the initial period of life a strong P can develop from a weak P, in other words, whether the strength of P changes.

Natural anti-P does not seem to occur frequently in human sera. Among a number of different animals in which large numbers of serum samples are easy to obtain in sufficient quantity (cow, sheep, hog), hogs showed the greatest productivity of natural anti-P agglutinin.

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RESULTS OF INVESTIGATIONS OF THE BLOOD GROUP
CHARACTERISTIC P, FROM THE
HYGIENIC INSTITUTE OF COLOGNE

P. Dahr

Translation of "Über die bisher im Kölner Hygienischen Institut
gewonnenen Untersuchungsergebnisse über das Blutmerkmal P."
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RESULTS OF INVESTIGATIONS OF THE BLOOD GROUP
CHARACTERISTIC P, FROM THE
HYGIENIC INSTITUTE OF COLOGNE*

P. Dahr

The impetus for publication of this report was the work by ^{**}/346 P. H. Andresen, from the Institute of Legal Medicine of the University of Copenhagen and is published in this journal, under the title: "Investigation of the blood group system P, determined by a strong isoagglutinin". In this article, Andresen reports that the detection of an anti-P agglutinin in human serum furnished him with the ability to embellish knowledge regarding this characteristic. I would like to state in advance that the communication of the investigative results by Andresen reports no new insight into the blood characteristics P. On the other hand, Andresen only hinted at investigations on the factor P, which have been done by my colleagues at the Hygienic Institute of Cologne for the last 2-1/2 years, and which did enrich our knowledge of the P-characteristic, especially concerning its mode of inheritance. Further, the proposal of Andresen to use P determination in passing judgement on cases of doubtful fatherhood, can only be based on the investigations made by the Hygienic Institute of Cologne. I would like to deal with investigative results made by Andresen and show that they do not /347 represent new insight, but rather confirmations of already well-known facts.

1. It is nothing new that the "irregular" agglutinins occurring in human serum, among which we find the anti-P agglutinin, must be considered to be the so-called "cold agglutinins" since their effectiveness is limited to low temperatures. Because of the not infrequent occurrence of such "irregular" agglutinins in human serum, one would have to anticipate rather frequent incidents during the transfer of blood of the same group, if these agglutinins would be as effective at higher temperatures (body temperature) as the isoagglutinins anti-A and anti-B.

2. In the course of investigating 506 blood samples, Andresen found 82.3% to be P-positive and 17.7% P-negative. He calls this finding a confirmation of the distribution ratio of 82% P-positive and 18%

*These investigations were conducted with means provided by the Society of Friends and Promoters of the University of Cologne.

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/Numbers in the margin indicate pagination of the original foreign text.

P-negative found in the white population by Landsteiner and Levine. He fails to mention the results of investigations made by myself and Zehner, which were published in 1941 and which dealt with 3530 investigations in Cologne, where we found 75% P-positive (= P) and 24% P-negative (= p).

3. By means of agglutination and absorption, Andresen determined that the amount of P-characteristic can vary in different individuals. This finding is not merely a confirmation of the fact originally found by Landsteiner and Levine, but also of my own results, published in this journal in 1939 (Vol. 97, p. 168). Andresen distinguishes between three classes of "P-intensity" which he designates P_1 , P_2 and P_3 . In 1939, I distinguished two classes of intensity, viz., "P-strong" and "P-weak", and on the basis of results of several family investigations, I mentioned the possibility that these different P-characteristics may exhibit a different mode of inheritance, similar as in the case of A_1 and A_2 , inasmuch as the P-strong trait would be dominant over P-weak. Furthermore, Andresen's division of the P-characteristic into three classes of intensity does not seem convincing, since in determining the agglutinability differences, which are said to be characteristic of the classes P_1 , P_2 and P_3 , the anti serum agglutinates the P_1 blood corpuscle up to a dilution of 1/64 (+), the P_2 blood corpuscles to a dilution of 1:32 (+) and the P_3 blood corpuscles to a dilution of 1:8 (+). Thus P_1 and P_2 are separated by a difference in agglutinability of only 1/2 degree of dilution, which, in my opinion, does not justify a differentiation. However, during absorption, the difference of two degrees of dilution is detectable, which more easily justified a differentiation. For establishing different degrees of intensity, there must be clear differences, which are detectable not only by means of absorption, but which are also evident from the agglutinability. Personally, I consider it possible that the varying strength of the P-characteristic in different individuals is due to the fact that there is yet another agglutinable characteristic (similar to the class MN in the MN system or to the blood group AB in the ABO system) whose gene is capable of combination with the gene for the P-characteristic. If we call this hypothetical agglutinogen X, the P-strong blood samples would be genetically homozygotic (PP), similar to the relatively strong M with the genetic configuration MM; the blood samples P-weak, on the other hand, would genetically be PX, similar to the relatively weak M in the blood class MN. If we assume such a "counterpart" to the agglutinogen P and its combination behavior regarding corresponding genetic traits, the observation made by Landsteiner and Levine, which stated that in marriages where both parents were P-weak, P-strong children could be found, we would find an easy explanation. In this case, the genetic

picture PX would appear as P-weak and in the children the genetic picture PP would appear as P-strong. I have been searching for some time on the grounds of this working hypothesis by using animal sera to find an agglutinin which would detect the hypothetical agglutinin X.

Andresen is of the opinion that, based on genetic investigations made to date, one is able to use P determination in certain cases to determine the possibility of a doubtful fatherhood, but that it is necessary to study the occurrence of the P-characteristic in the infants. On the basis of the determination of receptor strength by means of titration in 42 adults and 35 children down to the age of one year, he arrives at the conclusion that in infants, the receptor strength is general- /349 ly much lower. In adults, he finds a greater portion of strongly agglutinable P bloods and in children he finds relatively more P bloods which are less agglutinable than those which are strongly agglutinable. Without doubt, in an investigation with so few cases a small error can be significant, and the results of the investigation cannot be considered to be universally valid. In this context also, he fails to mention the investigations done by myself and Wiesener and published in 1940, which dealt with the blood characteristic P in neonates. At that time, we compared 1560 unselected adult blood samples with 300 unselected blood samples from neonates and found the following distribution ratio of P (= presence of P) and p (= lack of P):

Adults	Neonates
P = 82.8%	P = 84.3%
p = 17.2%	p = 15.7%

According to this result, we considered it improbable that an inherited P-characteristic would not be detectable in neonates. The second question we asked at that time was whether the characteristic P, found in neonates, changes in strength within the early period of life, and, if this is the case, when the final P-strength is reached. To answer this question, we used comparative determinations of the agglutination titer after 1, 2, 3, or more months, using the same anti-P serum and the same technique. In 14 of the children examined we found no change in the P-strength, but we thought the number of investigations to be too small to draw an accurate conclusion. Because of the war, these investigations could not be continued.

After these remarks pertaining to the conclusions drawn in Andresen's report, I would like to summarize the results of the P investigations conducted at the Hygienic Institute of Cologne, inasmuch as they have not already been mentioned.

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$A_1 P \dots 1859 = 76\%$,	$A_1 p \dots 693 = 24\%$
$A_2 P \dots 399 = 76\%$,	$A_2 p \dots 128 = 24\%$
$B P \dots 411 = 70\%$,	$B p \dots 176 = 30\%$
$O P \dots 1919 = 74\%$,	$O p \dots 653 = 26\%$
$AP P \dots 181 = 75\%$,	$AB p \dots 59 = 25\%$
$M P \dots 1273 = 73\%$,	$M p \dots 451 = 27\%$
$N P \dots 1068 = 74\%$,	$N p \dots 375 = 26\%$
$MN P \dots 2428 = 76\%$,	$MN p \dots 883 = 24\%$

GENETICS OF THE BLOOD CHARACTERISTIC P

Landsteiner and Levine already assumed that P was hereditary, in spite of the fact that the family investigations conducted at that time did not clearly reveal a definite mode of heredity. After futile attempts to obtain an anti-P serum from Landsteiner or elsewhere, in 1938, I found a very strongly effective anti-P agglutinin in pig serum. Landsteiner confirmed it to be anti-P, and I began my investigations in Cologne. These investigations have yet to be concluded. The opinion of Landsteiner and Levine concerning the heredity of the blood characteristic P was confirmed by means of investigation of 322 pairs of twins. 134 EZ pairs showed concordant behavior, whereas 188 ZZ pairs showed part concordance and part discordance:

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EZ = 134 pairs, of whom P:P = 123
p:p = 21
P:p = 0

ZZ = 188 pairs, of whom P:P = 126
p:p = 19
P:p = 43

It was soon clear from the family investigations that we could assume simple dominant heredity for the inheritance of P, with the gene "P" for the presence and "p" for the lack of the characteristic; P is dominant over p. The possible genetic pictures correspond to the observable picture as follows:

Genetic picture	Observable picture
PP	P = presence
Pp	P = presence
pp	p = lack

The investigations conducted by my colleagues Brinkmann, Hansen, Nussbaum, Offe, Wahlen, Weber and myself, which dealt with 563 families with a total of 2070 children, seem to confirm the assumed mode of heredity. Thus, from the different marriages, the following descendants are possible, or impossible, respectively:

Marriage	Possible children	Impossible children
P:P	P, p	-
P:p	P, p	-
p:p	p	P

In the families investigated so far, the findings with the children were as follows:

Parents	Children	
	P	p
P: P = 319	980	131
P:p = 194	505	277
p:p = 50	4	173

Thus, only 4 children contradict the correctness of the assumed mode of inheritance. In the case of 3 of these children, illegality was admitted and in the fourth case it is considered to be probable. Thus, the deviations found only represent apparent deviations from the assumed mode of heredity. /352

In the meantime, my colleague Berger is conducting further family investigations, the results of which will be published shortly.

Following publication of the heredity examinations on the blood factor P in the Hygienic Institute of Cologne, according to which the

mode of heredity of P, described above, is considered likely to be correct, the Hygienic Institute has been asked again and again, by courts of law confronted with cases of doubtful fatherhood, whether it would be possible to exclude the possibility of fatherhood on the basis of the P test. Personally, I hold the viewpoint that fatherhood must be considered to be highly unlikely, if, on the basis of the P test, exclusion is possible. However, exclusion is only indicated in cases where mother and defendant both have the factor p, and the child has the factor P.

Finally, a few remarks concerning:

THE DETERMINATION OF THE P-CHARACTERISTIC

The blood characteristic P can be detected with three different kinds of serum, viz., 1. Human serum, which, of course, must contain an anti-P agglutinin. The effectiveness of these sera is usually rather low, however, the serum found by Andresen seems to be stronger and hence useable.* 2. P detection is possible with some animals sera (especially from horses and pigs) which contain natural anti-P. The preparation of these sera was thoroughly described in my report: "Techniques of blood group and blood factor determination" Leipzig, G. Thieme, 1940. 3. P detection is possible by means of immune sera, which can be obtained by injecting P-containing human blood corpuscles into animals. My attempts to obtain such immune sera from rabbits, have, so far, always had negative results. Recently, I have begun similar experiments with guinea pigs, which may lead to success. Since the beginning of my investigations, I have exclusively used normal animal anti-P sera, obtained from horses or pigs, which contained a sufficiently strong anti-P agglutinin. I worked exclusively with the specimen holder method, according to which the mixture of a drip of anti-P serum and a 5% blood corpuscle suspension is stirred briefly with a small glass rod and then spread somewhat. After that, the slide or the glass plate is not moved for about two minutes, then one moves it with a swaying motion in order to accelerate and strengthen the reaction. The final reading is taken after 15 minutes. I prefer the specimen holder technique, and especially recommend the stirring and spreading of the mixture because, due to the low affinity of the animal anti-P agglutinins for human P agglutinogens, the reaction is accelerated by the spreading. Even weak P-characteristics are more easily detectable by using this method than with any other method with

* In the meantime, Prof. Werner Fischer (Berlin) has sent me a human serum which contains a very useable anti-P agglutinin.

which I am familiar. Several anti-P sera were always used for the P determination. I obtained suitable sera either from Prof. Dr. H. Schmidt (Marburg), or from Asst. Dr. Geiger (Eystруп) from the horse and pig farms in Marburg and Eystруп kept by the Behring factory, or from the pigs of the NSV pig breeding farms of Cologne. I brought one pig from the NSV with an especially useable anti-P agglutinin, using it in the Institute for serum donations as they were needed. During the numerous determinations with several anti-P sera from animals, I never achieved results that deviated qualitatively, and if strong enough sera were used, an accurate diagnosis of "P" = P-positive and "p" = P-negative was always possible. I have never observed transitional forms, i. e., between P and p such as Landsteiner and Levine found earlier, and in the presence of which a clear delineation of P /354 and p would undoubtedly be more difficult. In an earlier publication I remarked that these transitional forms of barely detectable P described by Landsteiner and Levine are apparently due to the use of human anti-P sera with weak anti-P agglutinins. Whether and how far qualitatively different P results can be reached in the investigation of a blood sample with anti-P agglutinins of different origin, I will only be able to test when I have succeeded in obtaining an immune sera whose agglutination results can be compared with those reached with standard animal sera, which are the only ones that I have used so far.

It would be very desirable if investigations into the heredity of P would be conducted elsewhere. I am willing to furnish ready-to-use anti-P serum to any interested colleague for the purpose of initial orienting investigations, especially for the detection of A, B and O blood without P, which are necessary for the purification of anti-P raw sera.

SUMMARY

A brief survey of the investigations into the hereditary blood factor P, conducted since 1939 in the Hygienic Institute of Cologne.

The number of blood samples tested for P, up to December 10, 1941, is 6478. Of these, 4769 (74%) had the characteristic P, whereas 1709 blood samples (26%) lacked the P-factor.

The presence of the P-factor is designated with "P" and the lack of it with "p".

The heredity of P was studied in 322 pairs of twins, and the mode of inheritance was studied in 563 families with a total of 2070 children. The results of the studies on twins confirm the heredity of P; the family

studies reveal a simple, dominant mode of inheritance of P. In four children we observed results contradicting the accuracy of the assumed mode of inheritance. In three of these cases, illegitimacy was admitted and in the other cases illegitimacy is highly probable.

On the basis of the results of the heredity studies, it is deemed justifiable to declare fatherhood highly unlikely if it can be excluded on the basis of the P determinations.

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Correction: In the meantime, other twin pairs could be investigated; the configuration is now the following: total studies number 383 pairs.

EZ = 157 pairs, of whom P:P = 141
p:p = 26
P:p = 0

ZZ = 226 pairs, of whom P:P = 149
p:p = 23
P:p = 54